

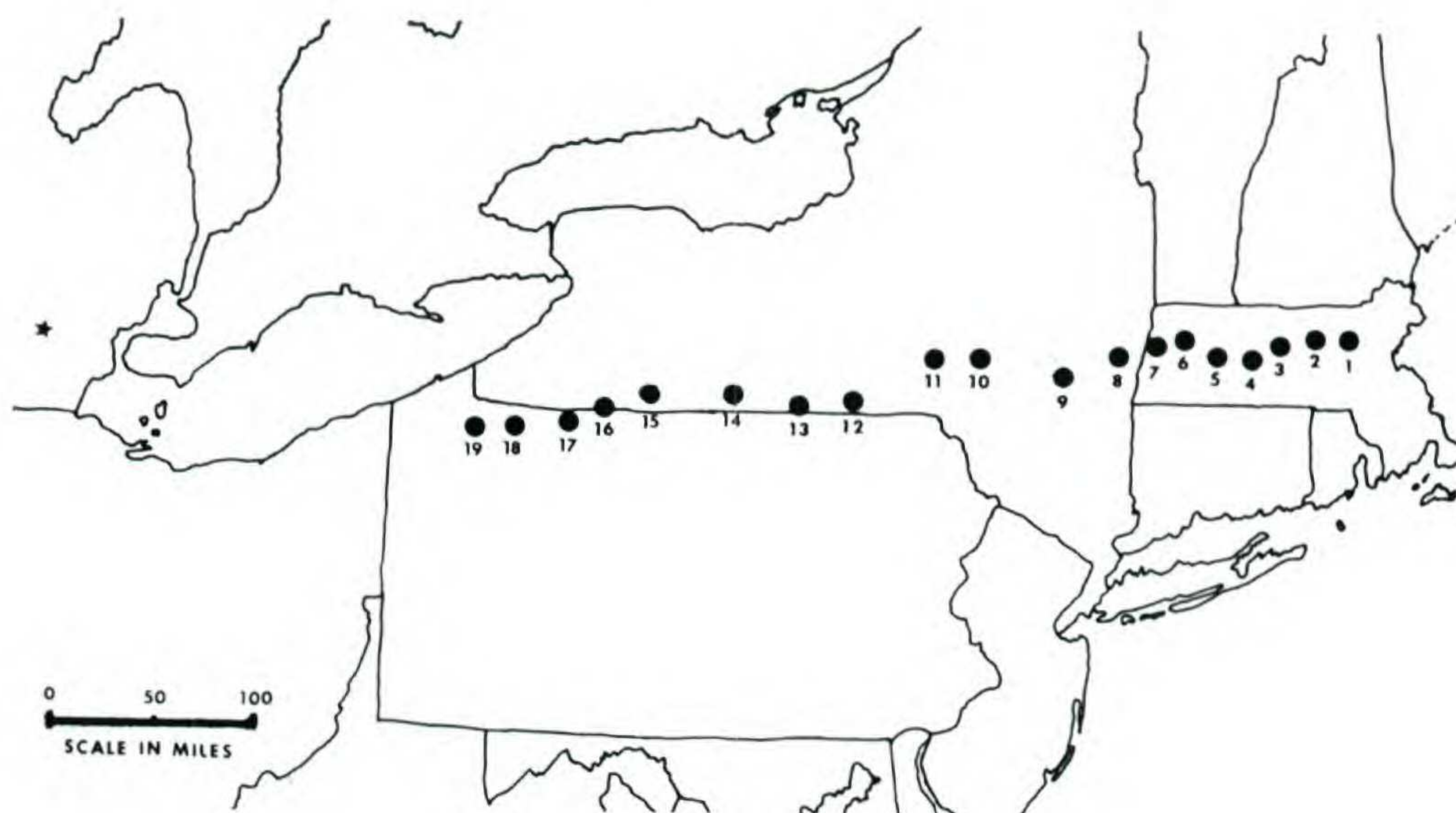
SOIL PREFERENCES AND VARIATION
IN FLAVONOID PIGMENTS
IN SPECIES OF ASTER

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A plant has certain environmental limits within which it can operate with greater or lesser success. The characteristics of the phenotype will determine the relative success of the plant. The environment is not static, but variable both in time and in space. For a species to occupy more than a point in time and in space, it has to have the ability to operate successfully under different environmental conditions. Two major mechanisms help the plant cope with a variable environment: (1) adjusting its phenotype and its metabolism through physiological and developmental responses so that the phenotype be as close to optimum as possible for the prevailing situation; (2) adapting genetically through the production of a great array of diverse genotypes and an excess of progeny, from which presumably only the best suited will grow to maturity.

The purpose of these studies is to determine the various facets involved in the adaptation of a species to conditions that vary in space and in time. The dilemma for the investigator is how to establish both the environmental factors that limit the distribution of a species, and also the phenotypical and genetical characteristics that make the distribution possible, given that neither the environment nor the morphological and genetical characteristics are constant in time and space. One classical technique is that of transplant experiments. Population samples are grown under a relatively uniform environment, and compared to one another. The differences that are found are presumably responsible for the capacity of the two populations to grow in different habitats. There are several problems in this method that will be discussed, but in general it has provided interesting information.

The genus *Aster* is widespread, common, and taxonomically difficult. In the northeastern United States there is



(See Collection Data on page 262)

ample evidence of repeated hybridization (Wetmore and Delisle, 1939; Uttal, 1962), as well as polyploidy (Van Faasen, 1963; Solbrig, 1967). Avers (1953a, b) was able to document both hybridization and polyploidy in the heterophylli group, and also some degree of chromosomal homology, crossing relationships and possible phylogeny. For this reason it was felt that this group would be amenable to more in depth ecological studies, than any other group of *Asters*.

MATERIALS AND METHODS

A transect was established nearly due west from Boston to the general area of the corner of the states of Pennsylvania, New York, and Ohio (fig. 1). Along this transect a population was sampled every twenty to fifty miles. The sample was restricted to populations belonging to the heterophylli group of *Aster* (Fernald, 1950; Avers, 1953b). Each sample consisted of at least ten plants and not more than twenty, and these were selected at random. The plants were brought to the Botanical Gardens of the University of Michigan in Ann Arbor, Michigan where they were broken into two clones and both halves were then planted in a uniform garden following a randomized design. In addition a soil

sample was gathered in a plastic bag at most of the localities and brought to the laboratory for analysis.

Soil Analysis

Soil samples were characterized as to pH, moisture retention capacity, nitrate content, and mechanical composition.

The hydrometer method was used for the mechanical analysis. Moisture retention was obtained using a low pressure ceramic plate extractor; readings at 0.8 atmospheres tension (12p.s.i.) and at 0.1 atmospheres were secured. The pH values were determined by mixing 10 grams of fresh soil and 25 ml of distilled water; readings were taken one and three hours after the mixing of the soil and the water. Soil nitrate content was determined following the phenoldisulphonic acid method (Metson, 1956).

Qualitative Studies of Pigments

The fresh leaves were diced and placed into 99% MeOH and 1% HCl in the dark for 24 hours. Only mature basal leaves were used during the study. The plant material was separated from the solvent and secondary compounds by filtration through Whatman No. 1 filter paper. Two hundred micro-liters of this extract was spotted onto either thin-layer plates or Whatman No. 3 MM chromatography paper (46 × 57 cm sheets). Due to the fact that better separations were obtained with the paper technique the majority of the work was done in this way. The chromatograms were developed (descending) using tertiary butyl alcohol, acetic acid, and water (3:1:1) in the first direction. Caution was exercised to insure that fresh solvent was utilized since the above solvent undergoes esterification reactions rapidly. After thorough drying, the chromatograms were again placed in the chromatocab and developed in the second dimension with 15% acetic acid and water. All the chromatography was done in an air-conditioned, dark room. The temperature was maintained as close to 21° C as possible.

The compounds were tentatively identified by their characteristics under both short-wave and long-wave ultra-

violet light, and by their behavior in the presence of ammonia vapor. Certain phenolic compounds (whitish or bluish in UV) and certain flavonoids (dark or yellowish in UV) appeared in all populations studied while other compounds appeared only off and on.

Quantitative Studies of Anthocyanins

The anthocyanin production studies were completed using leaf-discs from each plant. These were punched from the mature basal leaves with a cork borer (#6) and grown for seven days on a 0.1 M sucrose solution under constant light, at 20° C. The discs were sliced and extracted in a 1% HCl solution for a week on a shaker at approximately 6° C. The amount of anthocyanin was determined colorimetrically using the method of Thiman and Edmonson (1949).

RESULTS

A. Soil Analysis. The results of the soil analysis can be seen in table 1. Although the area traversed is one of varied soils, including some that are rich in silts and clays, all the populations sampled, regardless of species, grew in sandy or sandy-loam soils, with a neutral or more commonly acid pH, low organic matter and low nitrate concentration, although there was considerable variation in this last character. The uniformity of the soil type cannot be assigned to chance and must indicate a preference for this type of soil by the species sampled.

B. Secondary Compounds. Although there was variation in the kind and number of compounds present, in general there was no significant variation that could be correlated between populations. There was a great deal more physiological variation due to development of the plant and environmental variation within the experimental plot than variation due to genetical causes.

Table 2 summarizes the results of the analysis for all the populations investigated. Of the 25 compounds that were evident on the chromatographs, 6 were present in all 83 plants that were analyzed, 10 compounds were present in over 60 of the analyzed plants and 9 compounds were

truly variable. That is they were absent from entire populations and when present in a population often only some plants would show them. No compound was species specific.

Tests were run on both replicates of a clone, and in addition the same plants were tested at different times of the year. The results are summarized in table 3. It can be seen that the variations are relatively great. Still greater differences can be obtained if plants are grown in the greenhouse. When this is done, certain spots fail to show up on the chromatogram. A further source of variation is the technique itself. If a larger or lesser amount of extract is used, the number of spots can be sometimes varied accordingly.

C. *Anthocyanin Production.* The quantitative studies were restricted to measuring the total amount of anthocyanin that the species were capable of producing. The anthocyanin was identified as cyanidin. The object was to see whether (1) different species and populations varied as to the amount of anthocyanin they can produce, and (2) the variations were species specific or if they could be correlated with environmental factors. Preliminary studies (Solbrig, 1966) indicated that there were correlations between altitude above sea level and latitude where the populations grew and that there were also variations between species as to their anthocyanin production.

The results of the present study are summarized in table 4. It can be seen that indeed there are variations between populations and that they are not species specific. There is appreciable difference that can be ascribed to site. It should be remembered, however, that although there are differences in elevation between sites, all the populations grew at about the same latitude.

DISCUSSION

Uniform garden studies are designed to eliminate environmental differences between localities in the shaping of the phenotype. Any differences between phenotypes growing in a uniform environment can therefore be ascribed to genetic differences. It is conceivable, however, that dissimilar

Table 1. Analysis of 24 soil samples.

Pop. No.	Nitrate N ₂ .ppm/wt of fresh soil	Moisture % a-d soil	Moisture retained at 0.1 atmos. press. % o-d soil	Moisture retained at 0.8 atmos. press. % o-d soil	Dry loss on ignition % a-a soil	> 2 mm. % a-d soil	Coarse sand % a-d soil	Fine sand % a-d soil
426	264.9	4.0	67.9	48.1	21.8	3.9	28.0	26.8
427	89.35	1.8	47.8	26.9	13.8	3.2	25.2	22.6
428	111.6	1.4	24.0	13.9	10.8	25.8	71.8	11.4
430	99.2	2.2	44.2	16.1	6.6	2.0	25.1	29.5
431	5.875	1.5	40.9	19.5	6.4	7.3	28.8	32.7
432	41.6	0.8	26.9	9.6	6.2	15.1	54.0	20.2
433	31.54	1.6	48.0	29.2	12.0	9.6	23.8	13.8
434	2.3	—	—	—	—	—	—	—
435	80.0	—	—	—	—	—	—	—
436	17.6	1.4	16.9	8.6	5.0	31.8	55.4	20.6
439	79.0	2.2	40.6	25.9	8.9	19.5	29.4	15.6
440	14.75	1.2	17.6	8.4	3.7	36.6	62.0	13.4
441	212.65	1.4	41.3	23.6	11.8	32.6	45.2	10.4
442	227.4	0.8	33.6	22.9	8.2	8.9	39.0	25.8
445	140.76	2.6	34.0	18.2	3.7	14.9	22.5	25.1
444	49.8	1.4	34.5	20.4	8.0	18.7	27.7	21.7
445	80.5	0.8	37.9	19.7	7.6	52.6	22.9	20.1
446	20.0	—	—	—	—	—	—	—
447	50.6	1.2	37.6	22.1	8.4	27.0	19.7	16.7
448	45.6	2.2	40.9	29.6	9.3	8.1	5.3	9.5
449	41.65	1.4	44.3	24.2	8.8	33.8	16.1	14.3
450	89.225	—	—	—	—	—	—	—
451	34.0	—	—	—	—	—	—	—
452	35.5	0.8	30.2	16.8	9.0	26.8	37.0	15.0
X		1.616	37.32	2.125	8.947	19.91	33.63	19.22
s ²		0.6094	137.95	83.43	16.86	189.29	289.79	44.56
s		0.7806	11.745	9.134	4.106	13.76	17.023	6.675

Total sand % a-d soil	Silt % a-d sand	Clay % a-d soil	pH	Color		Soil type
				Hue	Chroma Value Munsell Chart	
54.8	36.8	7.2	5.15	10 Yr	2/2	Sandy loam/loam
47.8	42.0	10.2	4.45	10 Yr	4/2	Loam
83.2	11.0	5.8	4.72	2.5 Yr	4/2	Loamy sand
54.6	37.2	8.2	5.15	10 Yr	4/2	Sandy loam
61.5	27.0	11.2	5.72	2.5 Yr	5/2	Sandy loam
74.2	17.6	8.2	7.20	10 Yr	1/2	Sandy loam
37.6	45.8	1.66	6.20	10 Yr	5/2	Loam
—	—	—	6.43	5 Yr	4/3	—
—	—	—	7.37	2.5 Yr	6/2	—
76.0	14.8	9.2	7.21	5 Yr	5/2	Sandy/sandy loam
45.0	39.8	15.2	5.46	5 Yr	4/2	Loam
75.4	12.8	11.8	7.05	5 Yr	5/2	Sandy loam
55.6	30.2	14.2	5.80	7.5 Yr	4/2	Sandy loam
64.8	26.0	9.2	4.78	10 Yr	3/3	Sandy loam
47.6	39.6	12.8	6.92	10 Yr	3/1	Loam
49.4	29.0	21.6	6.89	10 Yr	4/3	Loam
43.0	30.8	17.2	5.82	5 Yr	4/2	Loam
—	—	—	6.58	10 Yr	3/1	—
36.4	40.4	23.2	6.50	10 Yr	5/2	Loam/clay/loam
14.8	46.8	29.2	5.52	7.5 Yr	4/2	Clay loam
30.4	46.4	23.2	7.11	10 Yr	3/2	Loam
—	—	—	5.01	10 Yr	3/2	—
—	—	—	5.54	10 Yr	6/2	—
52.0	31.8	16.2	6.38	10 Yr	5/2	Loam/sandy loam
52.85	32.36	14.23	6.04			
296.26	133.37	40.88	0.805			
17.21	11.549	6.394	0.8972			

Table 2
Summary of chromatographic analysis

Pop. Number	Spot No.																								
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
427 (8)	+	+	+	?	+	+	+	+	?	?	+	+	+	+	?	?	+	+	+	?	+	?	?	?	?
430 (4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	?	?	+	+	+	?	+	?	?	?
431 (9)	+	?	+	+	+	?	+	?	?	?	?	+	+	+	+	+	?	?	+	+	?	?	?	?	?
432 (8)	+	+	+	?	+	+	+	+	?	?	+	+	+	+	+	+	?	?	+	+	+	+	?	?	?
433 (8)	+	+	+	+	+	+	+	+	?	?	?	+	+	+	?	?	+	+	+	?	+	+	0	?	?
434 (8)	+	+	+	?	+	+	+	+	?	?	?	+	+	+	?	?	+	+	+	+	?	+	+	+	?
435 (3)	+	+	+	+	+	+	+	+	0	?	?	0	+	+	0	0	+	+	+	+	+	+	?	?	+
439 (8)	+	+	+	+	+	+	+	+	?	?	?	+	?	+	+	?	?	+	+	+	?	+	?	?	?
440 (7)	?	+	+	+	+	+	+	?	?	?	+	?	?	+	+	?	?	+	+	?	?	?	?	?	0
441 (1)	+	+	+	+	+	+	+	+	0	0	+	+	0	+	+	?	?	+	+	0	+	?	?	?	+
442 (2)	+	+	+	+	+	+	+	+	0	0	+	?	+	+	+	?	?	+	+	?	?	?	?	?	?
445 (1)	+	+	+	+	0	+	+	+	+	0	+	+	+	+	0	+	+	+	+	0	+	?	?	?	0
446 (2)	+	+	+	+	+	+	+	+	?	?	+	+	+	+	+	?	?	+	+	?	?	?	?	?	0
447 (5)	+	+	+	?	+	+	+	+	+	+	+	+	+	+	?	?	+	+	+	?	?	?	?	?	?
448 (2)	+	+	+	?	+	+	+	+	0	0	0	+	?	+	0	+	0	+	+	+	?	?	?	?	?
449 (1)	+	+	+	+	+	+	+	0	0	0	+	+	+	+	+	+	+	+	+	0	+	+	+	+	0
450 (3)	+	+	+	+	+	+	+	0	0	0	+	+	+	+	+	?	?	+	+	+	?	?	?	?	?
452 (2)	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	0	0	+	+	+	?	?	?	?	?
454 (2)	+	+	+	+	?	+	+	+	+	0	+	+	+	+	+	0	0	+	+	+	?	?	?	?	?
	I	I	P	I	I	I	P	P	I	V	V	I	I	P	I	V	I	P	P	P	V	V	V	V	V

+ all plants of the population contained the compound

? some plants of the population did and others did not contain the compound

0 no plants appeared to have the compound

I over 60 of the 83 plants tested showed the compound

P all 83 plants showed the compound

V variable compound

TABLE 3

Summary of temporal variation in leaf pigments

Spot No.

Pop. & Plant	Date Run	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
427-9	8/6/68	+	+	+	0	+	+	+	+	+	0	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+
427-9	9/12/68	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	+	+	0
432-3 Row 15	8/11/68	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+
432-3 Row 16	8/11/68	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	+
432-4 Row 15	8/11/68	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	+	+	+	+	+	+	+	+
432-4 Row 16*	8/11/68	+	+	+	+	+	+	+	+	+	+	0	+	+	+	0	+	+	+	+	+	+	+	0	+	0
432-4 Row 16*	8/11/68	+	+	+	0	+	+	+	+	+	+	0	+	+	+	0	+	+	+	+	0	+	+	0	+	+
439-4	8/1/68	+	+	+	+	+	+	+	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	0	+	+
439-4	10/13/68	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0

*Different leaves from the same plant

TABLE 4

Total Anthocyanins produced by 10 leaf discs

Pop.	Species	Col. Site	Antho. ave. value*
426	cordifolius	(1)	138
428	undulatus	(3)	124
429	cordifolius	(4)	352
430	cordifolius	(5)	129
431	cordifolius	(6)	59
433	cordifolius	(8)	141
434	cordifolius	(9)	148
435	ciliolatus	(9)	140
436	ciliolatus	(9)	62
444	lowreianus	(14)	150
447	cordifolius	(16)	55
448	cordifolius	(17)	98
449	cordifolius	(18)	169
450	cordifolius	(19)	93
451	cordifolius	(19)	65
454	cordifolius	(20)	169

*Readings in Klett units using #54 filter and standardized for volume.

genotypes will react to the same environment by producing similar phenotypes, the well known phenomenon of canalization. This problem to a certain degree puts into question some of the conclusions arrived at in any transplant experiment.

The most striking feature in this study is the uniformity of the populations, considering that we are dealing with four species and three levels of ploidy (9, 13, 36). All plants in their native environment grew in very similar soils. This indicates a definite preference by these species for loose sandy soils, a fact not previously established experimentally.

More interesting, however, from a taxonomic point of view are the studies on the secondary compounds. The seasonal and environmental variation found should be a fair warning to those attempting to use similar methods of chromatography of secondary compounds for taxonomic purposes using random field samples or herbarium samples. At least in the heterophylli *Aster* secondary compounds vary greatly during the growing season, and are also affected by the particular environment of the plant. The environment affects both the number as well as the intensity of spots produced.

It appears that during the evolution of the heterophylli *Aster* little or no differentiation has occurred in soil preference, at least in the N.E. United States. There seems to have been little change in the alcohol soluble secondary compounds also. There have been no species specific changes in the amount of anthocyanin that the various species produce.

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Collection Data

Sites

1. Mass., on Rte. 111. 4.7 miles N. of Jct with Rte. 110 Harvard Township. 67-426 *Aster cordifolius* (Solbrig 4046); *Aster cordifolius* (Solbrig 4047).
2. Mass., Hwy 31, 1 mile south of Hwy 12. Plants growing under oak, beech, and maple. 67-428 *Aster undulatus* (Solbrig 4048).
3. Mass., 5 miles east of Hardwick, under maple forest. 67-429. *Aster cordifolius* (Solbrig 4049).
4. Mass., Jct of 9 and 202. Growing under a maple-oak forest. 67-430. *Aster cordifolius* (Solbrig 4050).
5. Mass. Hwy 9, 1 mile east of Williamsburg, under an oak-maple forest. 67-431. *Aster cordifolius* (Solbrig 4051).
6. Mass., Hwy 9, 1.9 miles east of Windsor. Growing under a mixed hardwood-softwood forest, mostly at the more illuminated edges. 64-432. *Aster cordifolius* (Solbrig 4052).
7. Mass., Hwy 20, 8.1 miles west of Pittsfield, at the edge of a maple-oak forest. 67-433. *Aster cordifolius* (Solbrig 4053).

8. New York, Rte. 23, 1.2 miles east of East Windham. Plants growing in mixed maple, elm, and oak forest. 67-434. *Aster cordifolius* (Solbrig 4054); 67-435. *Aster ciliolatus* Lindl. (Solbrig 4055); 67-436. *Aster ciliolatus* Lindl. (Solbrig 4056); 67-437. *Aster acuminatus* Lindl. (Solbrig 4057); 67-438. *Aster acuminatus* Lindl. (Solbrig 4058).
9. New York, Hwy 10, 4.6 miles south of Stamford. Growing on an embankment under a few maples at the edge of a field. 67-439. *Aster cordifolius* L. (Solbrig 4059).
10. New York, Hwy 28, 6.1 miles east of the Jct with 7B. 67-440. *Aster cordifolius* (Solbrig 4060).
11. New York, on Hwy 7, 1.2 miles south of Riverside. Growing in a weedy area with goldenrods, brambles, rhus, etc. 67-441. *Aster cordifolius* (Solbrig 4061).
12. New York, Hwy 17, 8.7 miles east of Oswego. (Jct Hwy 283) Growing in a weedy area under a few maple trees. 67-442. *Aster cordifolius* (Solbrig 4062).
13. New York, Hwy 17 east of Elmira (3.2 miles east of Chemniz) Growing on the edge of a disturbed woods. 67-443. *Aster cordifolius*. (Solbrig 4063); 67-444. *Aster lowreianus* (Solbrig 4064); 67-445. *Aster laevis* (Solbrig 4065).
14. New York, Hwy 17, 1.6 miles east of Addison. Growing at the edge of a beech-oak forest. 67-446. *Aster cordifolius* (Solbrig 4066).
15. New York, west outskirts of Wellsville. Growing under maples and *Crataegus*. 67-447. *Aster cordifolius* (Solbrig 4067).
16. New York, 3 miles south of the village of Whitehouse. Growing in woodland with a border of mixed maple and oak with many introduced shrubs. 67-448. *Aster cordifolius* (Solbrig 4068).
17. Pennsylvania, on Hwy 770, 4.3 miles east of the Jct with 59. Growing in a secondary forest of oak, maple, beech, and hickory. 67-449. *Aster cordifolius* (Solbrig 4069).
18. Pennsylvania, Hwy 27, 2 miles west of Pittsfield. Growing at the edge of an open field, in a ditch, and at the forest edge respectively. 67-450. *Aster cordifolius* L. (Solbrig 4070); 67-451. *Aster sagitifolius* (Solbrig 4071); 67-455. *Aster acuminatus* (Solbrig 4073).
19. Pennsylvania, Hwy 77, 1 mile south-west of Jct Hwy 8. Growing in small woods at the edge of the road. 67-454. *Aster cordifolius* (Solbrig 4074).